

NUTRIENT UTILIZATION AND GROWTH RESPONSES OF THE FRYs OF THE AFRICAN HYBRID CATFISH (*CLARIAS GARIEPINUS* X *HETEROBRANCHUS BIDORSALIS*) TO INORGANIC PHOSPHORUS SUPPLEMENTS.

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Abstract

*Sixteen triplicate diets each supplemented with either of 4 inorganic phosphorus (P) sources (monosodium phosphate, monopotassium phosphate, monocalcium phosphate and dicalcium phosphate) at 0.40%, 0.60%, 0.80% or 1.20% level were fed to fry's of the African hybrid catfish (*Clarias gariepinus* x *Heterobranchus bidorsalis*) (weighing 2.80 ± 0.11 g) at 5% body weight in aquaria for 70 days. A non-P-supplemented diet and a purified diet served as controls. Growth of fish was monitored by weekly protein intake, protein efficiency ratio, nitrogen metabolism, feed conversion, specific growth rate and gain or loss of tissue protein. Proximate composition of the diet was determined. The results showed that the parameters varied significantly ($P < 0.05$) among (a) the 18 test diets, (b) the sources of inorganic phosphorus and (c) the duration of experiment. The results also indicated that monosodium phosphate was a better source of inorganic phosphorus supplement in the hybrid's diets than other sources. The hybrids responded nutritionally better to control diets than the P-supplemented diets.*

Key words: Inorganic dietary phosphorus, African hybrid catfish, monosodium phosphate.

Introduction

Studies by fish nutritionists on dietary phosphorus (P) uptake of various fish species indicated that the channel catfish (*Ictalurus punctatus*) fingerlings raised under laboratory conditions and fed semi-purified diets containing graded levels of P required 0.33% (Wilson *et al.*, 1982) to 0.40% (Lovell, 1978) of available P for maximum growth. Hence, commercially produced feeds have been formulated to contain 0.4 to 0.5% available P. When animal protein such as fish meal was used as a feed component, the high level of P content in it results in excessive P content in commercial feeds. Therefore, the application of such a feed in aquaculture systems leads to release of large amounts of P in water, thereby increasing the P loads in effluents (Kendra, 1991) or in the sediments surrounding cage culture systems (Kelly, 1993).

Probably, the most effective way to minimize phosphorus discharge to the environment is to reduce the amount of dietary P to a level as low as possible while still maintaining optimal fish health and performance (NRC, 1993). It was recommended that the inclusion of P supplied from plant ingredients that are relatively low in available P and supplementing with inorganic P sources could be a remedial measure to minimize phosphorus loads. Various research works have recognized dietary P as an important factor in soft and hard tissue formation and their maintenance (Reinhart & Mahan, 1986). Because P is an expensive dietary nutrient, dietary levels are generally formulated to achieve optimum growth rate.

Working on the phosphorus budgets for channel catfish ponds receiving diets with different phosphorus concentrations, Gross *et al.* (1998) showed that the uptake of phosphorus by pond bottom soil and conversion to phosphorus in fish flesh were responsible for the major losses of phosphorus in channel catfish ponds. They maintained that P concentrations in pond water and phytoplankton activity were not strongly influenced by P levels in diets, and there was no influence of P inputs in diets and amounts of P in pond at

harvest. It was further suggested that there was little direct benefit of low phosphorus diets in reducing P concentrations and phytoplankton abundance in channel catfish pond.

Little work has been done on the phosphorus requirements of the African catfish and their hybrids as to the amount of dietary phosphorus absorbed by them. Such study would help catfish farmers know how to supplement diets with inorganic phosphorus as to reduce phosphorus loads in ponds. This study was therefore designed to investigate the nutrient utilization and growth responses of the fry of the African catfish hybrid fed diets supplemented with inorganic phosphorus. The criteria for evaluation were weekly protein intake, protein efficiency ratio, nitrogen metabolism, feed conversion, specific growth rate and gain/loss of tissue protein.

Materials and Methods

Advanced fry of the African catfish hybrid *Clarias gariepinus* (♂) Burchell, 1882 x *Heterobranchus bidorsalis* (♀) Geoffroy St. Hillarie 1809 were obtained from an outlet of a private fish hatchery, Aqua fish Nigeria Limited, Awka, Nigeria and transported in six 50-liter plastic containers to the Research Laboratory at Ebonyi State University, Abakaliki. Eight hundred and ten of these hybrid fry (mean weight, 2.80 ± 0.11 g) were randomly stocked in 54 plastic baths each with a volume of 25 litres and acclimated for 7 days. They were fed on a maintenance ration of chicken starter diets at 1% body weight per day.

The experiment was designed to have triplicate treatments of 16 diets supplemented with four inorganic phosphorus (P) groups (A, B, C, D) and two controls made up of a non-phosphorus supplemental diet and a purified diet (Table 1). The inorganic phosphorus sources used were: monosodium phosphate (MSP), monopotassium phosphate (MPP), monocalcium phosphate (MCP) and dicalcium phosphate (DCP). These were included at 0.40%, 0.60%, 0.80% and 1.20% in the diets (Table 2). The fish were then fed twice daily (8 a.m. and 4 p.m.) at 5% body weight per day for 70 days. Temperature in the plastic baths was measured with the aid of a maximum-minimum thermometer. The water pH was determined with a pH meter (Model Ph -J-201-1) while fish sampling for weight was done every 7 days using a Mettler balance (Model PL 210) and feed allowance for the subsequent 7 days adjusted in accordance with 5% body weight of fish.

The proximate composition of the test diets for moisture was determined by drying the samples in a convection oven at 105° C to constant weight, as well as the crude protein which was determined using the microkjeldahl technique, gross energy by bomb calorimetry, ash by combustion in muffle furnace at 600° C and fibre by the asbestos method in accordance with Windham (1995). Crude lipid (ether extract (EE)) was determined by soxhlet extraction method (Folch et al., 1957), and nitrogen-free extract (NFE) was computed by difference, thus: $NFE = 100 - (\% \text{ ash} + \% \text{ crude protein} + \% \text{ crude fibre} + \% \text{ crude lipid} + \% \text{ moisture contents})$ (Marshall, 1974).

The weekly protein intake (PIW) was derived from the relationship of feed intake and % protein in the diet, thus: $PIW = \text{feed intake (g)} \times \% \text{ protein in diet}$; the protein efficiency ratio (PER) was the mean weight gain (g) per protein intake (g); while the maintenance nitrogen metabolism (NM) was calculated using Dabrowski (1977) method as follows:

$$NM = (0.549) \frac{(a + b)h}{2}$$

where a = initial weight of fish (g); b = final weight of fish (g), and h = experimental period in days.

The food conversion ratio (FCR) was the relationship of mean weight gain (g) per feed intake (g); while the specific growth rate (SGR) was according to Brown (1957) method, thus:

$$SGR (\% \Delta WD^{-1}) = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$$

where W_2 = weight in time T_2 days; W_1 = weight in time T_1 days; Δ = change in weight (g), and D = days.

The gain or loss of tissue protein (GLP) was calculated from the difference of the gain or loss of fish tissue protein after every 7 days. The Analysis of Variance (ANOVA) was used to statistically test the treatment means of the collected data for significant differences while the least significant difference (LSD) was used to partition the differences (Steel & Torrie, 1980).

Results

Tables 3, 4, 5, and 6 show the results obtained in this study. The water temperature ranged between 27°C and 28°C and the pH was 6.80 ± 0.15 during the experiment. The nutritional performance parameters determined for the fish varied significantly ($P < 0.05$) among the 18 test diets, the sources of inorganic P supplementation and the experimental duration.

Of the P-supplemented diets, the proximate composition of the fish indicated that fish fed MSP diets deposited more crude protein ($P < 0.05$) in their body tissue than the other three test diets. The control diets however, deposited better CP values than any of the P-supplemented diets. The values of the crude lipid of the fish increased in the order: MSP (7.05%) > MPP (5.95%) > MCP (4.83) > DCP (3.50%). The control diets, CD (8.69%) and PD (8.66%) were higher than the P-supplemented diets. Similarly, the ash content of treatment diets from MSP was lower than those of control diets (Table 3).

Table 4 shows the weekly protein intake of the experimental fish. The results indicate that the protein intake of the test fish resulting from the various crude protein sources increased in the order: MSP (1.29 %) > MCP (1.28 %) > MPP (1.8 %) = DCP (1.8 %). Weekly protein intake of the control diets (1.13 % and 1.01%) were less than those of the P-supplemented diets. Differences in weekly protein intake of fish due to the various CP sources were significant ($P < 0.05$).

The protein efficiency ratio (PER) of fish fed the P-supplemented diets showed that Diet 4 (1.20% MSP) recorded the highest PER (0.28) (Table 4). This is lower than that of any of the control diets (0.33 and 0.32) (Table 4). There was a progressive decrease of PER from day 7 (0.98) to day 70 (0.05) (Table 5).

The maintenance nitrogen metabolism of the fish ranged from 28.84 g-day with diet 14 (0.60% CP) to 36.05 g-day with diet 17 (a control diet) (Table 4). The effect of the supplemental phosphorus sources on NM showed that MSP (33.36 g-day) > MCP (31.96 g-day) > DCP (30.78 g-day) > MPP (30.68 g-day). The NM of fish fed the control diet (36.05 g-day) was higher than any of the P-supplemented diets (Table 4). Maintenance nitrogen metabolism increased progressively from day 7 (8.50 g-day) to day 70 (50.03 g-day) (Table 5).

The MSP diet (3.51) gave the best and significantly different FCR, followed by MCP (3.72) which is not significantly different ($P > 0.05$) from DCP (4.04) and lastly MPP (4.11) ($P < 0.05$) (Table 4). The FCR recorded with the control diets (1.82 and 1.65) were significantly ($P < 0.05$) better than the FCR of any of P-supplemented diets (Table 4). The food conversion ratio values increased as the experiment progressed from day 7 to day 70 (Table 5).

The specific growth rate of the fish ranged from 1.21% per day when the fish were fed with diet 11 (0.80% MCP) to 1.44 % per day when the fish were fed with diet 18 (a control diet) (Table 4). The effect of the P-supplemented diets on the SGR also indicated that but for diet 18, there was no significant difference ($P > 0.05$) in SGR among the fish fed the other diets. As recorded for the PIW, PER, NM and FCR; there were significant differences ($P < 0.05$) on the effect of the 18 test diets, the sources of P supplementation and the

experimental duration (days) on the SGR of fish.

The gain or loss of tissue protein ranged from -9.41 % with the fish fed diet 2 (0.60 % MSP) to 3.01% with the fish fed purified diet (Table 4). Among the supplemental P sources, there was no significant difference in GLP ($P > 0.05$). Each of the control diets effected better gain of tissue protein than any of the P-supplemented diets (Table 4). The gain of tissue protein was most at day 7 (0.70 %) followed by day 14 (0.52 %) and day 35 (0.31 %), respectively, while protein loss was recorded at day 21 (-0.10%) and day 49 (-8.14%) (Table 5).

Discussion

Fish fed with the MSP diet apparently consumed more but not significantly different protein (1.29 %) than any of the P-supplemented diets (MCP, 1.28 %; MPP, 1.18 %; and DCP, 1.18 %) (Table 4) ($P < 0.05$). The range of PIW values (1.03 % - 1.47 %) obtained during this study compared favourably with the 1.20% - 1.72% reported for *Tilapia aurea* fingerlings (Wu & Jan, 1977) although the workers used purified diets as their feed source.

The result on protein efficiency ratio recorded for MSP-supplemented diets indicated that fish fed with MSP diet (0.27) performed nutritionally better than any of the other P-supplemented diets and the controls. The variability in the PER was possibly due to the levels of P supplementation and the inorganic phosphorus sources of the diets. However, PER in this study increased with increasing levels of P-supplementation which indicated that increase in phosphorus level increased PER values in all the diets (Table 4). The range of PER in this study (0.23 to 0.33) compared favourably with that obtained by Faturoti *et al.* (1986) (0.17 - 0.35) who fed non-P-supplemental diets of 27 - 40% CP to the African catfish (*Clarias lazera*) fry for 8 weeks. These PER values, however, varied with the range (0.69 - 1.26) reported for the catfish (*C. gariepinus*) broodstock (Faturoti *et al.*, 1992) and 2.25 for most warm water fishes (Balogun *et al.*, 1992). The differences in the various PER values could be ascribed to age, species differences, dietary type and experimental conditions. Cowey *et al.* (1974) reported that the metabolic rate of fish was high at young stage and much of the ingested protein is used as a source of energy. Additionally, the period of fast growth of fry is between 6 – 8 weeks (Coche & Bianchi, 1979) implying that the 4 to 14 weeks fry used in this study fell within the range of fast growth. Barring species differences therefore, the 4 to 14 weeks old fry of the African hybrid catfish had more efficient utilization of the ingested protein for their metabolic activities.

Unlike the PER, the NM of fish did not show any sequential increase with increasing phosphorus levels from 0.40 to 1.20% P (Table 4). The higher NM value recorded for the control diet (36.05) relative to the P-supplemented diets implied that phosphorus concentration in the P-supplemented diets could have suppressed nitrogen metabolism in the experimental fish. This is reflected in other growth parameters such as PER, CP and SGR.

That the food conversion ratio increased as the experiment progressed implied that time affected the ability of the fish to convert a unit gram of diet consumed to a unit gram of fish flesh. The range of FCR values, 3.51 - 4.04 (for the P-supplemented diets) and 1.65 - 1.82 (for the control diets) of this study contrasted with results of other workers for some species of fish and agreed with the results of some other workers for other species of fish. For the temperate catfish, Robinson *et al.* (1996) recorded better FCR values (1.43 - 1.45) for channel catfish (*Ictalurus punctatus*) fed isonitrogenous diets supplemented with 0.26 - 1.30% MSP while the FCR values (1.60 - 1.90) recorded for the same species (Eya & Lovell, 1997) which were lower than the FCR values for the P-supplemented diets. Results of the present study compares quite favourably with findings of previous works reviewed above. For non-silurid fishes, the FCR of fish fed the control diets compared favourably with 1.60 - 1.90 recorded for red drum (*Sciaenops ocellatus*) but contrasted with 3.51 - 4.04 (Donald and

Robinson, 1987) for P-supplemented diets and 2.50 - 3.30 for MSP-supplemented diets fed to *Oreochromis niloticus* fingerlings for 12 weeks (Robinson *et al.* (1987).

The inconsistency in the specific growth rate of fish with increasing percent P-supplementation (Table 4) is not understood and it was at variance with the increasing trend of PER as the P-supplementation level increased. However, higher SGR values recorded with the MSP diet when compared with MCP, MPP and DCP diets corroborates with the higher values recorded for PER, NM and FCR with the MSP diet. This implied that the MSP diet was a better choice for supplementing African hybrid catfish diets with inorganic phosphorus than MCP, MPP and DCP diets. Nevertheless, the fish fed the control diets gave better nutritional responses as measured from PER, FCR and NM than any of the P-supplemented diets. This implies that the inorganic phosphorus concentrations at the rates used were above the optimum level required for the hybrid catfish fry. This agrees with the findings of Coloso *et al.* (2003) that beyond 0.88% available phosphorus, growth of the rainbow trout did not improve.

The gain or loss of tissue protein followed the similar inconsistent trend as with the SGR as the inorganic P level increased (Table 4). However, the magnitude of loss of tissue protein when the P-supplemented diets were fed to fish was closely related to the average crude protein content of fish. This trend further agrees with the suggestion that based on the results from this study, monosodium phosphate was the best choice for dietary inorganic P-supplementation for the African hybrid catfish fry.

CONCLUSION AND RECOMMENDATION

From the results of this study it is inferred that monosodium phosphate was a better source of inorganic phosphorus supplement in the diets of the fry of the African hybrid catfish (*Clarias gariepinus* x *Heterobranchus bidorsalis*) than other inorganic phosphorus sources. The hybrids responded nutritionally better to control diets than the P-supplemented diets. It is recommended that monosodium phosphate be used when inorganic phosphorus is needed in the African hybrid catfish diet.

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Table 1 Gross Composition of Inorganic Phosphorus (P) Supplemented Experimental Diets and Control Diets Fed the African Hybrid Catfish Fry for 70 days.

Ingredient	Diet					
	A	B	C	D	Control	Purified
Yellow maize	9.81	9.55	9.29	9.07	-	-
Soyabean meal	54.76	54.89	54.84	54.86	-	-
Fish meal	10.95	10.96	10.97	10.97	-	-
Palm oil	5.00	5.00	5.00	5.00	0.25	0.3
Salt	0.25	0.25	0.25	0.25	-	-
Vitamin mix ¹	0.60	0.60	0.60	0.60	-	-
Calcium & P-free mineral mix ²	1.80	1.80	1.80	1.80	1.80	1.80
% P as MSP, MPP, MCP or DCP ³	0.40	0.60	0.80	1.20	0.00	0.00
Casein	-	-	-	-	10.32	33.00
Dextrin	-	-	-	-	54.68	35.00
Corn starch	-	-	-	-	10.94	20.00
Cod liver oil	-	-	-	-	5.00	3.00
Carboxymethyl cellulose	-	-	-	-	0.60	3.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

¹Vitamin mix provided the following constituents diluted in cellulose (mg kg⁻¹ of diet): thiamin, 10; riboflavin, 20; pyridoxine, 10; flacin, 5; pantathenic acid, 40; choline chloride, 3000; niacin, 150; vitamin B₁₂, 0.06; retinyl acetate (500,000 iu g⁻¹), 6; menadione-N-bisulphate, 80; inositol, 400; biotin, 2; vitamin C, 200; ethoxyquin, 200; alphatocopherol, 50; cholecalcipherol (1,000,000 IU g⁻¹).

²Contained as g kg⁻¹ of premix, FeSO₄. 7H₂O, 5; MgSO₄. 7H₂O, 132; K₂SO₄, 329.90; KI, 0.15; Na₂Cl₂, 45; Na₂SO₄, 44.88; AlCl₃, 0.15; CoCl₂. 6H₂O, 5; CuSO₄.5H₂O, 5; NaSeO₃, 0.11; MnSO₄.H₂O, 0.7; and Cellulose, 380.97.

³MSP = monosodium phosphate, MPP = monopotassium phosphate, MCP = monocalcium phosphate, DCP = dicalcium phosphate

Table 2 Dietary Phosphorus Contents and Levels of Inorganic Phosphorus (P) Supplementation in Experimental Diets Fed to African Hybrid Catfish Fry for 70 days.

Ingredient (%)	Diets				
	A	B	C	D	Control
Yellow maize	0.12	0.12	0.12	0.12	0.12
Soyabean meal	0.15	0.15	0.15	0.15	0.15
Fish meal	1.90	1.90	1.90	1.90	1.90
Blood meal	0.17	0.17	0.17	0.17	0.17
Inorganic P-supplementation with either MSP, MPP, MCP or DCP ¹	0.40	0.60	0.80	1.20	0.00
Carboxymethyl cellulose	0.80	0.60	0.40	0.00	1.20
Total	2.74	2.94	3.14	3.54	2.34

¹MSP = monosodium phosphate, MCP = Monocalcium phosphate, P = Inorganic phosphorus, MPP = monopotassium phosphate, DCP = dicalcium phosphate.

Table 3 Proximate Composition (%) of Inorganic Phosphorus-supplemented Diets fed to African Hybrid Catfish Hybrid Fry for 70 days¹.

Ingredient	Diet ²				Control ³				Statistics ⁴	
	A, MSP	B, MPP	C, MCP	D, DCP	CD	PD	Overall mean	SE	LSD	
Crude protein (CP)	15.28 ^a	13.96 ^b	14.13 ^b	13.47 ^c	19.21 ^d	19.00 ^d	14.75	0.19	0.42	
Ether Extract (EE)	7.05 ^a	5.95 ^b	4.83 ^c	5.38 ^d	8.69 ^e	8.66 ^e	5.70	0.25	0.55	
Ash (AS)	2.55 ^a	1.85 ^b	2.13 ^c	2.04 ^c	3.59 ^d	4.31 ^e	2.34	0.10	0.22	
Moisture Content	70.12 ^a	68.46 ^b	65.36 ^c	66.12 ^d	66.34 ^d	65.53 ^f	67.16	1.14	0.56	
Crude Fibre	0.67 ^a	0.62 ^a	0.64 ^a	0.66 ^a	0.62 ^a	0.63 ^a	0.64	.009	0.10	
Nitrogen-free Extract (NFE)	4.33 ^a	9.16 ^b	12.91 ^c	12.15 ^c	1.55 ^a	1.87 ^a	7.00	1.12	3.14	
Total	100.00	100.00	100.00	100.00	100.00	100.00				

¹Mean values in a row followed by different superscripts are significantly different (P > 0.05).

²Diets supplemented with either MSP = monosodium phosphate, MPP = monopotassium phosphate, MCP = monocalcium phosphate, or DCP = dicalcium phosphate.

⁴SE = standard error, L.S.D = Least significant difference.

Table 4 Mean Growth Performance of the African Hybrid Catfish Fry Fed Different Inorganic Phosphorus-Supplemented (P) Diets¹.

Diet ²	% P	Growth Parameter ³					FCR	SGR (%ΔWD ⁻¹)	GLP (%)
		PIW (%)	PER	NM (days)	(g)				
MSP diets									
Diet 1	0.40	1.31	0.26	31.88	4.33	1.33	-0.80		
Diet 2	0.60	1.21	0.26	32.43	3.52	1.30	-9.41		
Diet 3	0.80	1.17	0.27	34.06	3.23	1.41	-8.65		
Diet 4	1.20	1.47	0.28	35.09	2.95	1.33	-8.69		
Mean		1.29 ^a	0.27 ^a	33.36 ^a	3.51 ^a	1.34 ^a	-6.89 ^a		
MPP diets									
Diet 5	0.40	1.18	0.23	30.08	4.94	1.34	-7.20		
Diet 6	0.60	1.03	0.24	28.97	4.02	1.26	-7.33		
Diet 7	0.80	1.09	0.26	30.96	3.84	1.36	-7.20		
Diet 8	1.20	1.43	0.26	32.70	3.65	1.31	-6.88		
Mean		1.18 ^{ab}	0.25 ^b	30.68 ^b	4.11 ^b	1.32 ^a	-7.15 ^a		
MCP diets									
Diet 9	0.40	1.20	0.25	30.82	3.71	1.23	-6.53		
Diet 10	0.60	1.17	0.25	30.44	3.77	1.26	-6.69		
Diet 11	0.80	1.24	0.25	32.66	3.60	1.21	-6.37		
Diet 12	1.20	1.45	0.26	33.93	3.80	1.33	-6.54		
Mean		1.27 ^{ab}	0.25 ^b	31.96 ^b	3.72 ^{ab}	1.26 ^b	-6.51 ^a		
DCP diets									
Diet 13	0.40	1.17	0.24	29.48	4.32	1.27	-7.87		
Diet ²	% P	Growth Parameter ³					FCR	SGR (% D ⁻¹)	GLP (%)
		PIW (%)	PER	NM (g days)					
Diet 14	0.60	1.03	0.26	28.84	3.74	1.30	-7.77		
Diet 15	0.80	1.12	0.25	30.71	3.49	1.28	-7.70		
Diet 16	1.20	1.40	0.27	34.07	4.61	1.40	-7.30		
Mean		1.18 ^{ab}	0.26 ^b	30.78 ^b	4.04 ^{bc}	1.31 ^a	-7.66 ^a		
Control diets									

Diet 17	0.00	1.13 ^b	0.33 ^c	36.05 ^c	1.82 ^c	1.30 ^a	1.82 ^b
Diet 18 (purified)	0.00	1.01 ^c	0.32 ^c	31.66 ^b	1.65 ^c	1.44 ^c	3.01 ^b

¹Mean values in the same column followed by different superscripts are significantly different ($P > 0.05$).

²Diets supplemented with either MSP = monosodium phosphate, MPP = monopotassium phosphate, MCP = monocalcium phosphate, or DCP = dicalcium phosphate.

³PIW = Weekly Protein intake; PER = Protein efficiency ratio; NM = Nitrogen metabolism; FCR = Food Conversion ratio; SGR = Specific growth rate;

GLP = Gain or loss of tissue protein.

⁴SE = standard error.

⁵L.S.D = Least significant difference.

Table 5 Effect of Duration (Days) on the Mean Growth Performance Values¹ of the African Hybrid Catfish Fry Fed Phosphorus-supplemented Diets.

Growth parameters	Duration (Days)										Statistics		
	7	14	21	28	35	42	49	56	63	70	SE ²	LSD ³	
Weekly protein intake (PIW) (%)	0.20 ^a	0.44 ^b	0.66 ^c	0.84 ^d	1.05 ^e	1.29 ^f	1.56 ^g	1.69 ^h	1.88 ⁱ	2.56 ^j	0.009	0.024	
Protein efficiency ration (PER)	0.98 ^a	0.47 ^b	0.32 ^c	0.26 ^d	0.20 ^e	0.16 ^f	0.07 ^g	0.07 ^h	0.06 ^h	0.05 ⁱ	0.001	0.002	
Nitrogen metabolism (NM) (g day)	8.50 ^a	14.02 ^b	19.64 ^c	25.47 ^d	31.57 ^e	37.13 ^f	41.72 ^g	43.97 ^h	47.31 ⁱ	50.03 ^j	0.009	0.025	
Food Conversion ration (FCR)	0.38 ^a	0.79 ^b	1.16 ^c	1.46 ^d	1.84 ^e	2.22 ^f	5.62 ^g	6.34 ^h	7.17 ⁱ	9.13 ^j	0.001	0.003	
Specific growth rate (SGR) (% D ⁻¹)	3.77 ^a	2.51 ^b	1.72 ^c	1.42 ^d	1.25 ^e	0.91 ^f	0.28 ^g	0.42 ^h	0.48 ⁱ	0.37 ^j	0.001	0.002	
Gain or loss of protein (GLP) (%)	0.70 ^a	0.52 ^b	-0.10 ^c	0.06 ^d	0.31 ^e	0.24 ^f	-8.14 ^g	0.22 ^h	0.15 ⁱ	0.12 ^j	0.015	0.037	

¹ Mean values in the same row followed by different superscripts are significantly different (P < 0.05).

² S.E. = Standard error of mean,

³ LSD = Least significant difference.